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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
FERNANDEZ, SUSAN EMILY	
ART UNIT	**PAPER NUMBER**
1651	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/621,485	MUECKLER ET AL.	
	Examiner	Art Unit	
	Susan E. Fernandez	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 December 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-15 and 30-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-15 and 30-32* is/are rejected.

7)☒ Claim(s) *1* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on December 2, 2005, has been entered.

Claims 1, 3-15, and 30-32 are pending and are examined on the merits.

Claim Objections

Claim 1 objected to because of the following informalities: At line 8, Claim 1 recites "(iv)," though the claim also recited "(iv)" at line 7. It is suggested that the recitation "(iv)" be replaced with "(v)". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 3-8, and 30-32 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, claim 1 recites obtaining a membrane fraction and a cytoplasmic fraction from insulin responsive cell which had been incubated in the presence of insulin. Though the disclosure provides support for obtaining a membrane fraction from cells incubated at a cold temperature and exposed to insulin (page 20, lines 1-4), the disclosure states that the cytosol fraction is obtained from basal cells, i.e. unstimulated cells (page 20, lines 4-5). See also page 19, lines 31-33. Therefore, the disclosure does not provide support for obtaining a cytoplasmic fraction from the insulin responsive cells treated in the manner recited in steps (a) and (b) of claim 1. This is a new matter rejection.

Additionally, the recitation "PI 3-kinase is activated, said PDK1 is activated, said PDK2 is activated" in step (e) of claim 1 lacks support in the disclosure. Furthermore, it is respectfully noted that the asserted support given in the Remarks filed on December 2, 2005 are not persuasive. For instance, the asserted support on page 22, lines 16-20 of the specification does not expressly disclose activation of PI 3-kinase. Additionally, step (c) of claim 1 requires PDK2 activity or PDK1 activity in the membrane or cytoplasmic fraction, therefore, it is unclear how PDK2 and PDK1 are activated in step (e) if they are already active. It is unclear whether any of the steps following step (c) had inactivated PDK1 or PDK2 which would allow for their activation in step (e) to occur. This is a new matter rejection.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3-8, and 30-32 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is rendered indefinite by the recitation "ice-cold" since the metes and bounds of the term are unclear. Applicant's arguments filed December 2, 2005 have been fully considered but they are not persuasive. While noted that the term "ice-cold" is commonly used in the art, the term fails to delineate subject matter encompassed or not encompassed by the claim. Moreover, the applicant has not provided sufficient support for the assertion that the term "ice-cold" is understood by those skilled in the field to describe the temperature of solutions maintained on ice at approximately 0-4°C. With respect to the same language appearing in other patents, each patent stands alone based on the prosecution history. It is suggested that the term "ice-cold" be deleted from claim.

Additionally, claim 1 is indefinite because it is unclear how PDK1 and PDK2 can be activated in step (e) considering step (c). Specifically, step (c) of claim 1 requires PDK2 activity or PDK1 activity in the membrane or cytoplasmic fraction, therefore, it is unclear how PDK2 and PDK1 are activated if they are already active. It is unclear whether any of the steps following step (c) had inactivated PDK1 or PDK2 which would allow for their activation in step (e).

Thus, claims 1, 3-8 and 30-32 are rejected under 35 U.S.C. 112, second paragraph.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al. in view of Hill et al. (Methods Enzymol., 2002, 345: 448-463), Campbell (Biology, 3rd edition, 1992, Benjamin/Cummings Publishing Co., Inc., page 104), Vanhaesebroeck et al., Alessi et al., and Cross et al.

As discussed above, Wijkander et al. discloses the homogenization of rat adipocytes in order to obtain supernatants referred to as "cytosol fractions" and pellets referred to as "membrane fractions". The membrane fractions consist of various membranes, including plasma membranes. The cytosol and membrane fractions are present in a homogenization buffer that lacks chloride ions. A protein kinase B assay was performed on these fractions as described on

page 21521 under "Protein Kinase Assay", wherein a mixture consisting of ATP and 40 mM $MgCl_2$ is added. Figure 2 provides results obtained from these assays, noting the effect of stimulating cells with insulin prior to obtaining cytosol and membrane fractions. Membrane fractions were also combined with cytosol fractions and a kinase assay was performed of this mixture for both control and stimulated cells.

Wijkander et al. does not expressly disclose treating the membrane fraction (which contains plasma membrane) with a high salt solution, thus obtaining a salt-extracted plasma membrane fraction and an aqueous fraction which is desalted. Furthermore, it does not disclose combining the salt-extracted plasma membrane fraction with the desalted aqueous fraction, the cytoplasmic fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride. Additionally, Wijkander et al. does not expressly disclose that the protein kinase B (PKB) is activated by having a phosphorylated threonine residue and a phosphorylated serine residue, or specifically, a phosphorylated threonine residue at position 308 of SEQ ID NO:1 and a phosphorylated serine residue at position 473 of SEQ ID NO:1. Finally, Wijkander does not show that the activated protein kinase B is capable of phosphorylating a GSK3.

Hill et al. discloses that "membrane translocation is an important event in the stimulation of PKB activity, thus, in addition to monitoring the phosphorylation status and kinase activity, it is also desirable to determine the subcellular localization of PKB in response to different stimuli" (page 458, last paragraph through page 459, first paragraph). Furthermore, Hill et al. states that "preparation of subcellular fractions enriched in the plasma membrane allows the comparison of the relative proportion of membrane-bound and cytosolic PKB before and after stimulation"

(page 460, second paragraph). Preparation of a crude plasma membrane fraction is outlined on pages 461 and 462, wherein buffers used comprise less than 145 mM chloride. The paragraph under "Analysis of Subcellular Fractions" on page 462 indicates that the crude plasma membrane pellet is mixed with NP-40 lysis buffer (page 452) which comprises 50 mM Tris-HCl and 120 mM NaCl in order to remove insoluble material. Thus the crude plasma membrane pellet is in a solution comprising 170 mM chloride.

Campbell discloses that enzymes are "sensitive to salt concentration" and that "most enzymes cannot tolerate extremely saline (salty) solutions" (page 104, last paragraph).

Vanhaesebroeck et al. discloses that the phosphorylation of Thr308 in PKBα is "enhanced over 1000-fold in the presence of lipid vesicles containing low amounts of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2..." (page 565, first column, second paragraph). PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 correspond to PIP3 and PI(3,4)P2, respectively (page 561). The requirement of including PIP3 and PI(3,4)P2 for enhanced phosphorylation is supported by *in vitro* experimentation (page 565, first column, second paragraph, last sentence).

Alessi et al. discloses that "the full activation of PKBα *in vitro* requires the phosphorylation of Ser473 as well as Thr308" (page 266, first sentence).

Cross et al. discloses that Akt (PKB according to the examined application, page 1, third paragraph) phosphorylates GSK3 (page 789, last paragraph).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to mix the membrane fraction obtained in Wijkander et al. with the NP-40 lysis buffer as described in Hill et al. Furthermore, since this buffer would extract the plasma membrane, it would have been obvious to a person of ordinary skill in the art to desalt the

aqueous fraction obtained. It would have been obvious to combine the resulting fractions with all other ingredients described in Wijkander et al. It would have been obvious to add a phosphatidylinositol phosphate, such as PIP3 and/or PI(3,4)P2, to the combined fraction mixture in order to activate protein kinase B. Additionally, it would have been obvious to conclude that the protein kinase B activated through *in vitro* experimentation in Wijkander et al. would have required the phosphorlyation of a serine and a threonine residue. If the PKB corresponds to SEQ ID NO:1, it would have been obvious that the phosphorylated residues required for PKB activation are the residues at positions 308 and 473. Furthermore, it would have been obvious to conclude that the activated PKB could phosphorylate GSK3.

One of ordinary skill in the art would have been motivated to solubilize the membrane fraction in the high salt NP-40 lysis buffer in order to remove insoluble material that may interfere with kinase activity or protein concentration assays. The aqueous fraction obtained through the salt-extraction would have required desalting for its use in a reaction mixture because Campbell shows that protein activity can be detrimentally affected by salt solution concentration. It would have been obvious to add a phosphatidylinositol phosphate, such as PIP3 and PI(3,4)P2, because Vanhaesebroeck et al. emphasizes that PIP3 and PI(3,4)P2 are "the lipids that are crucial for the activation of PKB". Thus one of ordinary skill in the art would have been motivated to add PIP3 and PI(3,4)P2 in order to enhance PKB activation. Alessi et al. establishes that phosphorylation of both a serine and a threonine residue is required for *in vitro* activation of PKB, thus it would have been obvious that this would have been required in order to practice the Wijkander experiments. According to the first sentence on page 14 of the application under examination, PKBα corresponds to the kinase as depicted in SEQ ID NO:1. If

the particular PKB to be activated is PKBα, it is evident from Alessi et al. that Ser473 and Thr308 would have been phosphorylated. Additionally, it is clear from Cross et al. that the activated PKB could have the ability to phosphorylate GSK3.

Additionally, none of the references above expressly disclose that the plasma membrane fraction comprises of PDK2 activity, that the cytoplasmic fraction comprises PDK1 activity, or that the desalted aqueous fraction comprises PDK2 activity. However, since the references render the steps of claim 9 obvious, and since Alessi et al. demonstrates that PDK1 and PDK2 are found in cells and participate in PKB activation (see page 267, Figure 7), PDK1 and PDK2 would are inherently present in the fractions just as was found in the instant disclosure. M.P.E.P. § 2112 reads, "The claiming of a new use, new function or unknown property which is inherently present in the prior art does not necessarily make the claim patentable." Thus, a holding of obviousness is clearly required.

Applicant's arguments have been fully considered but they are not persuasive. In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). As discussed above, PDK1 and PDK2 activities are inherently present in the cell fractions, and indeed, would have been resolved into distinct fractions, since the references teach the steps recited in the claims under examination.

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al., Hill et al., Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. as applied to claims 9-15 above, and further in view of Bauer et al.

As discussed above, Wijkander et al., Hill et al., Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. render claims 9-15 obvious.

These references do not teach the application of their methods to other insulin-responsive cells besides adipocytes.

Bauer et al. discloses that islet cells are insulin-responsive cells (abstract).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to apply the methods used in Wijkander et al. to other insulin-responsive cells, including muscle cells, liver cells, and islet cells.

One of ordinary skill in the art would have been motivated to do this because Wijkander notes that studies are performed with rat adipocytes because there is need for information about insulin regulation of PKB from "insulin-responsive target tissues such as liver, muscle or adipose tissue" (page 21520, second column, second paragraph). One of ordinary skill in the art would therefore have been motivated to try the listed tissues in order to obtain more information about PKB regulation. Experimentation would include islet cells which Bauer indicates are insulin-responsive and resemble other insulin-responsive cells. There is a reasonable expectation of success that the methods applied to adipocytes would translate well when applied to other cells.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on

combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained. It is respectfully noted that the general teaching of Bauer et al. is provided in order to indicate that islet cells are insulin-responsive cells, which are cells of interest by the Wijkander study. Thus, a holding of obviousness is clearly required.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan E. Fernandez whose telephone number is (571) 272-3444. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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